

Supplementary Materials for

Antisense oligonucleotide therapy in a humanized mouse model of *MECP2* duplication syndrome

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Supplementary Materials

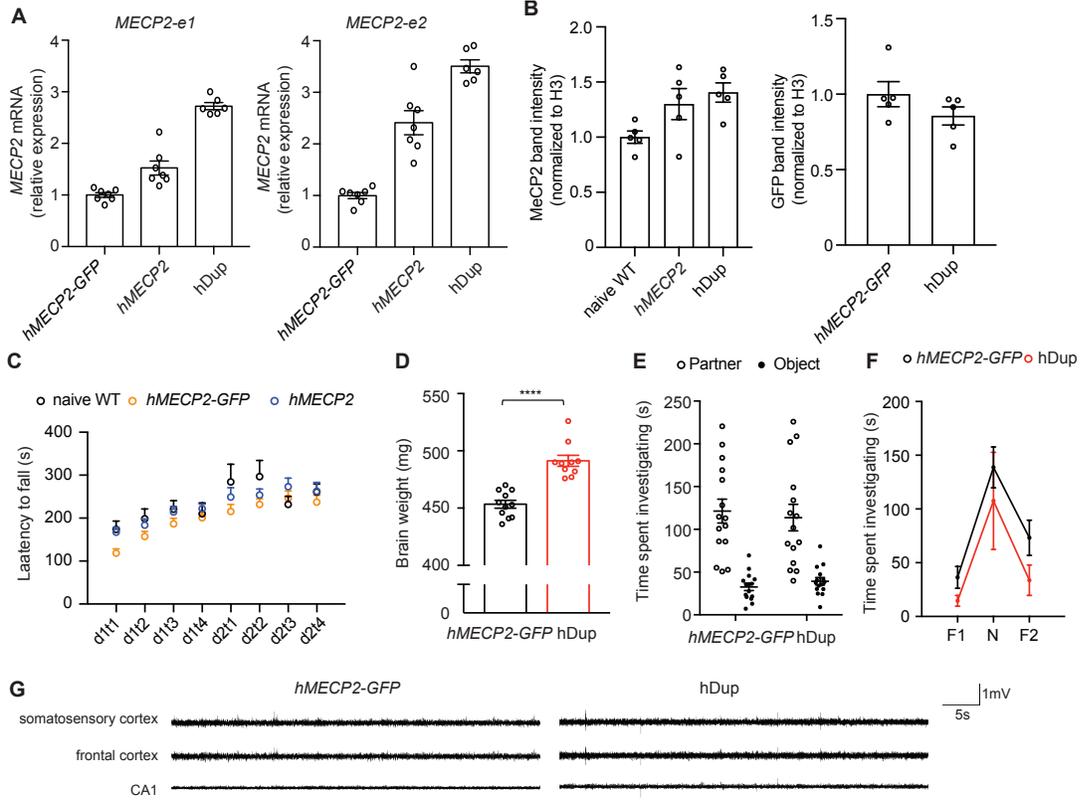


Fig. S1. Generation and characterization of a humanized *MECP2* duplication mouse model.

(A) RT-qPCR with primers for two isoforms of *MECP2* mRNA shows MeCP2 expression levels in the cortex of the humanized mouse model. **(B)** Quantification of the MeCP2 band with anti-MeCP2 antibody and the GFP band with anti-GFP antibody among four groups. (N=5) **(C)** Rotarod assay measures the motor function of naïve WT mice, *hMECP2*-GFP and *hMECP2* mice. d1t1, day 1/trial 1. **(E)** Three-chamber social interaction test on the humanized MDS mouse model at 8 weeks of age. **(F)** Partition test on the humanized MDS mouse model at 8 weeks of age. **(D)** Humanized MDS mouse model has greater brain weight at 8 weeks of age. **(G)** Humanized MDS mouse model has normal EEG. All data were analyzed by two-tailed *t*-test, except for the rotarod test that was analyzed by two-way ANOVA with repeated measures. Data are presented as mean \pm s.e.m. **P* < 0.05; ** *P* < 0.01; ****P* < 0.001; *****P* < 0.0001.

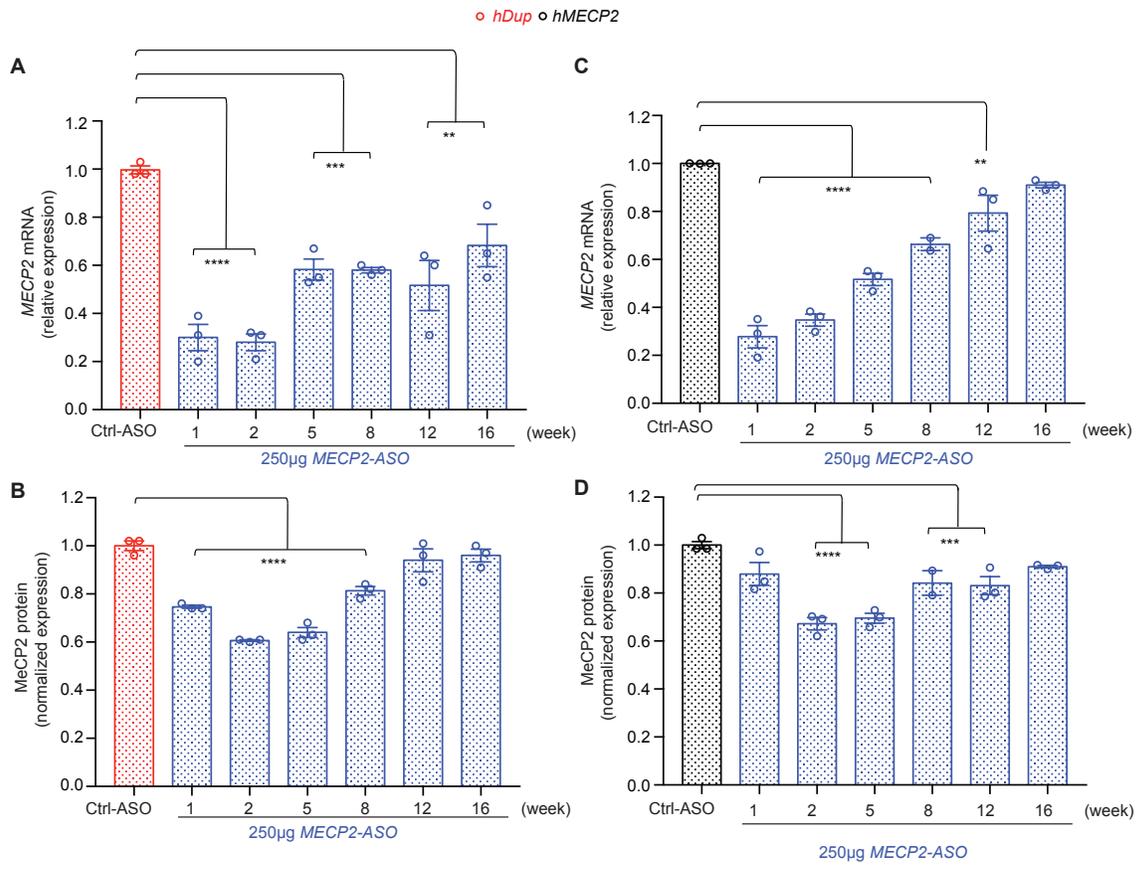


Fig. S2. Pharmacodynamics of 250µg of *MECP2*-ASO treatment. Post-treatment **(A)** *MECP2* mRNA levels by RT-qPCR in hDup mice; **(B)** MeCP2 protein levels in hDup mice; **(C)** *MECP2* mRNA levels by RT-qPCR in *hMECP2* mice; **(D)** MeCP2 protein levels in *hMECP2* mice. (cortex, n=3). All data were analyzed by one-way ANOVA followed by Fisher's LSD post hoc test. Data are presented as mean ± s.e.m. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

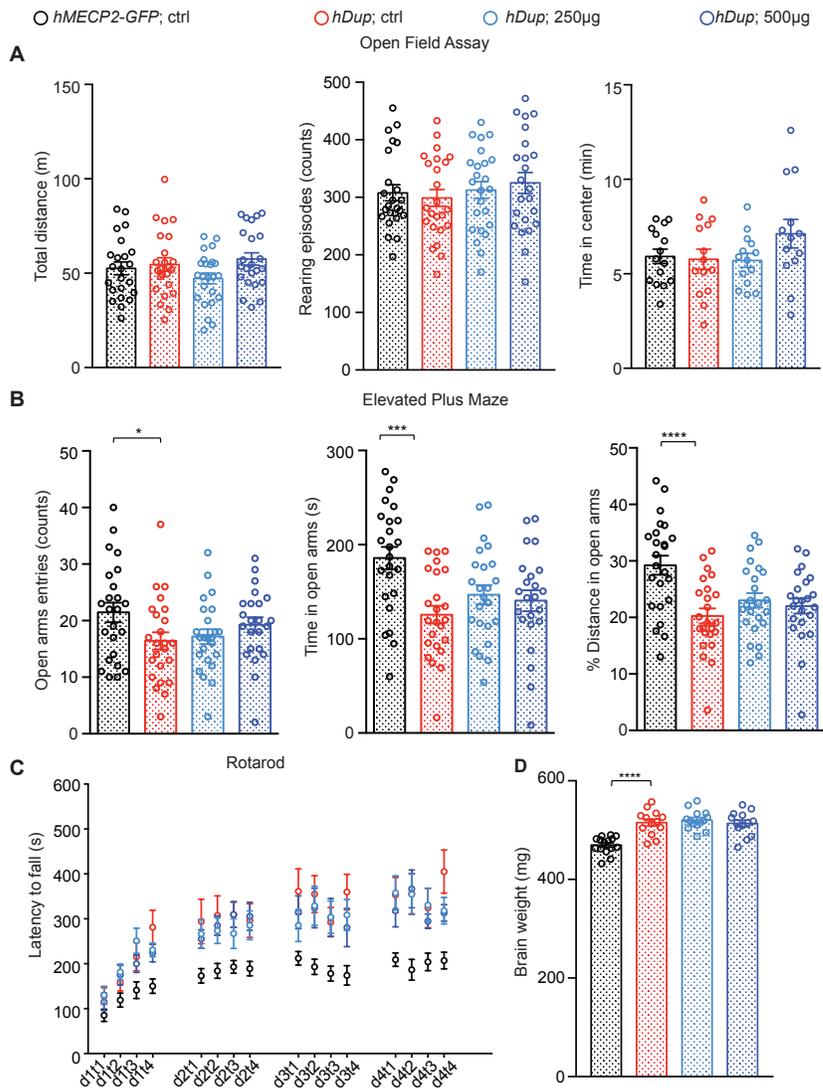


Fig. S3. *MECP2*-ASO treatment does not rescue abnormal behavior in humanized *MECP2* duplication mice at an early time point. (A) Activity of 13-week-old mice in the open field assay, 5 weeks after ASO treatment. **(B)** Anxiety-like behavior in the elevated plus maze test, 5 weeks after ASO treatment. **(C)** Brain weight of mice 12 weeks after ASO treatment. **(D)** Motor coordination of 14-week-old mice in the accelerating rotarod, 6 weeks after ASO treatment. d1t1, day 1/trial 1. All behavioral data were analyzed by one-way ANOVA followed by Fisher's LSD post hoc test. Rotarod test was analyzed by two-way ANOVA with repeated measures. n = 15 for *hMECP2-GFP*; ctrl group; n = 13 for *hDup*; ctrl group; n = 14 for *hDup*; 250 μ g *MECP2*-ASO group; n = 13 for *hDup*; 500 μ g *MECP2*-ASO group. Data are presented as mean \pm s.e.m. * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001. *hMECP2-GFP*; ctrl - *hMECP2-GFP* mice injected with control-ASO. *hDup*; ctrl - *hDup* mice injected with control-ASO. *hDup*; 250 μ g - *hDup* mice injected with 250 μ g *MECP2*-ASO. *hDup*; 500 μ g - *hDup* mice injected with 500 μ g *MECP2*-ASO

Table S1

All two-group comparisons performed with t-test; all multi-group comparisons performed with One-Way ANOVA with Fisher's LSD correction			
Figure	measurement	groups	individual P value
1B	<i>MECP2</i>	WT vs h <i>MECP2</i>	9.52E-06
		WT vs hDup	2.50E-06
1G	time in open arms	hGFP vs hDup	0.0002
	entries into open arms	hGFP vs hDup	0.0155
	distance in open arms	hGFP vs hDup	2.46E-05
1I	d2t1	hGFP vs hDup	0.0044
	d2t2	hGFP vs hDup	0.0003
	d2t3	hGFP vs hDup	0.0056
	d2t4	hGFP vs hDup	0.02
2C	Hypo	ctrl ASO vs 250µg ASO	3.93E-06
	Amy	ctrl ASO vs 250µg ASO	5.12E-04
	Str	ctrl ASO vs 250µg ASO	4.37E-05
	Ctx	ctrl ASO vs 250µg ASO	3.11E-07
	Hipp	ctrl ASO vs 250µg ASO	3.93E-06
	Ob	ctrl ASO vs 250µg ASO	1.99E-07
	Bs	ctrl ASO vs 250µg ASO	2.83E-06
	Cb	ctrl ASO vs 250µg ASO	4.54E-05
2D	Hypo	ctrl ASO vs 250µg ASO	0.0045
	Amy	ctrl ASO vs 250µg ASO	0.0311
	Str	ctrl ASO vs 250µg ASO	0.0158
	Ctx	ctrl ASO vs 250µg ASO	5.90E-07
	Hipp	ctrl ASO vs 250µg ASO	1.42E-05
	Ob	ctrl ASO vs 250µg ASO	9.29E-07
	Bs	ctrl ASO vs 250µg ASO	1.16E-06
3A	<i>MECP2</i>	hDup;ctrl ASO vs hDup; 150µg ASO	7.50E-07
		hDup;ctrl ASO vs hDup; 250µg ASO	1.03E-09
		hDup;ctrl ASO vs hDup; 500µg ASO	7.09E-11
3C	MeCP2	hDup;ctrl ASO vs hDup; 150µg ASO	0.0435
		hDup;ctrl ASO vs hDup; 250µg ASO	1.50E-06
		hDup;ctrl ASO vs hDup; 500µg ASO	1.14E-07
3D	IFN-γ	hDup-ctrl vs. hDup-250µg	0.0265
		hDup-ctrl vs. hDup-500µg	0.012

	<i>MECP2</i>	hDup-ctrl vs. hDup-250µg	0.007
		hDup-ctrl vs. hDup-500µg	0.0457
4A	<i>MECP2</i>	hDup-ctrl vs. hDup-1wk	6.45E-08
		hDup-ctrl vs. hDup-2wk	1.59E-07
		hDup-ctrl vs. hDup-5wk	0.0012
		hDup-ctrl vs. hDup-8wk	0.0262
		hDup-ctrl vs. hDup-12wk	0.0141
		hDup-ctrl vs. hDup-16wk	0.1361
4C	<i>MeCP2</i>	hDup-ctrl vs. hDup-1wk	0.0449
		hDup-ctrl vs. hDup-2wk	3.89E-05
		hDup-ctrl vs. hDup-5wk	4.40E-05
		hDup-ctrl vs. hDup-8wk	0.0086
		hDup-ctrl vs. hDup-12wk	0.1876
		hDup-ctrl vs. hDup-16wk	0.4475
4E	<i>AW551984</i>	hDup-ctrl ASO vs. hGFP-Ctrl ASO	2.18E-05
		hDup-ctrl ASO vs. hDup-1wk	0.344
		hDup-ctrl ASO vs. hDup-2wk	0.0001
		hDup-ctrl ASO vs. hDup-5wk	0.0002
		hDup-ctrl ASO vs. hDup-8wk	0.0006
		hDup-ctrl ASO vs. hDup-12wk	0.0002
		hDup-ctrl ASO vs. hDup-16wk	0.0003
	<i>Npbwrl</i>	hDup-ctrl ASO vs. hGFP-Ctrl ASO	1.50E-06
		hDup-ctrl ASO vs. hDup-1wk	0.003
		hDup-ctrl ASO vs. hDup-2wk	0.0013
		hDup-ctrl ASO vs. hDup-5wk	0.0005
		hDup-ctrl ASO vs. hDup-8wk	0.0001
		hDup-ctrl ASO vs. hDup-12wk	3.01E-05
		hDup-ctrl ASO vs. hDup-16wk	4.29E-05
	<i>Gdf11</i>	hDup-ctrl ASO vs. hGFP-Ctrl ASO	0.0059
		hDup-ctrl ASO vs. hDup-1wk	0.1156
		hDup-ctrl ASO vs. hDup-2wk	0.1497
		hDup-ctrl ASO vs. hDup-5wk	0.0217
		hDup-ctrl ASO vs. hDup-8wk	0.0143
		hDup-ctrl ASO vs. hDup-12wk	0.1803
		hDup-ctrl ASO vs. hDup-16wk	0.3958
	<i>Efemp1</i>	hDup-ctrl ASO vs. hGFP-Ctrl ASO	0.0435
		hDup-ctrl ASO vs. hDup-1wk	0.0221
		hDup-ctrl ASO vs. hDup-2wk	0.2213
		hDup-ctrl ASO vs. hDup-5wk	0.0306

		hDup-ctrl ASO vs. hDup-8wk	0.0813
		hDup-ctrl ASO vs. hDup-12wk	0.0993
		hDup-ctrl ASO vs. hDup-16wk	0.0679
	<i>Crh</i>	hDup-ctrl ASO vs. hGFP-Ctrl ASO	0.0706
		hDup-ctrl ASO vs. hDup-1wk	0.0317
		hDup-ctrl ASO vs. hDup-2wk	0.7065
		hDup-ctrl ASO vs. hDup-5wk	0.2326
		hDup-ctrl ASO vs. hDup-8wk	0.3747
		hDup-ctrl ASO vs. hDup-12wk	0.6057
		hDup-ctrl ASO vs. hDup-16wk	0.8626
	<i>Tsc22d3</i>	hDup-ctrl ASO vs. hGFP-Ctrl ASO	0.0119
		hDup-ctrl ASO vs. hDup-1wk	0.7308
		hDup-ctrl ASO vs. hDup-2wk	0.151
		hDup-ctrl ASO vs. hDup-5wk	0.0068
		hDup-ctrl ASO vs. hDup-8wk	0.0034
		hDup-ctrl ASO vs. hDup-12wk	0.0004
		hDup-ctrl ASO vs. hDup-16wk	0.0008
5A	rearing episodes	hDup-ctrl ASO vs. hGFP-ctrl ASO	0.0005
		hDup-ctrl ASO vs. 250µg	0.3744
		hDup-ctrl ASO vs. 500µg	0.0113
5B	context	hDup-ctrl vs. hGFP-ctrl	0.0011
		hDup-ctrl vs. hDup-250µg	0.6217
		hDup-ctrl vs. hDup-500µg	0.0052
	cued	hDup-ctrl vs. hGFP-ctrl	2.05E-13
		hDup-ctrl vs. hDup-250µg	0.1748
		hDup-ctrl vs. hDup-500µg	0.070
5C	Rotarod	D1	
		hDup-ctrl vs. hGFP	0.1942
		hDup-ctrl vs. hDup-250ug	0.8977
		hDup-ctrl vs. hDup-500ug	0.6469
		D2	
		hDup-ctrl vs. hGFP	0.0152
		hDup-ctrl vs. hDup-250µg	0.195
		hDup-ctrl vs. hDup-500µg	0.1854
		D3	
		hDup-ctrl vs. hGFP	0.0014
		hDup-ctrl vs. hDup-250µg	0.0081
		hDup-ctrl vs. hDup-500µg	0.0107
		D4	

		hDup-ctrl vs. hGFP	0.0001
		hDup-ctrl vs. hDup-250µg	0.001
		hDup-ctrl vs. hDup-500µg	0.0003
5D	open arms entries	hDup-ctrl ASO vs. hGFP-Ctrl ASO	0.0018
		hDup-ctrl ASO vs. hDup-250µg	0.9443
		hDup-ctrl ASO vs. hDup-500µg	0.5263
	% distance in open arms	hDup-ctrl ASO vs. hGFP-Ctrl ASO	0.0008
		hDup-ctrl ASO vs. hDup-250µg	0.6307
		hDup-ctrl ASO vs. hDup-500µg	0.7492
5E	<i>Npbwrl</i>	hDup-ctrl ASO vs. hGFP-Ctrl ASO	0.0011
		hDup-ctrl ASO vs. hDup-250µg	0.7014
		hDup-ctrl ASO vs. hDup-500µg	0.0066
	<i>AW551984</i>	hDup-ctrl ASO vs. hGFP-Ctrl ASO	0.0064
		hDup-ctrl ASO vs. hDup-250µg	0.2934
		hDup-ctrl ASO vs. hDup-500µg	0.0245
	<i>Crh</i>	hDup-ctrl ASO vs. hGFP-Ctrl ASO	0.0222
		hDup-ctrl ASO vs. hDup-250µg	0.1906
		hDup-ctrl ASO vs. hDup-500µg	0.0051
	<i>Tsc22d3</i>	hDup-ctrl ASO vs. hGFP-Ctrl ASO	0.0282
		hDup-ctrl ASO vs. hDup-250µg	0.0109
		hDup-ctrl ASO vs. hDup-500µg	0.0187
	<i>Efemp1</i>	hDup-ctrl ASO vs. hGFP-Ctrl ASO	0.0027
		hDup-ctrl ASO vs. hDup-250µg	0.013
		hDup-ctrl ASO vs. hDup-500µg	0.0085
	<i>Gdf11</i>	hDup-ctrl ASO vs. hGFP-ctrl ASO	0.0125
		hDup-ctrl ASO vs. hDup-250µg	0.8441
		hDup-ctrl ASO vs. hDup-500µg	0.0033